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Original article

Analysis of Urinary Organic Acid Profile in Clinically Suspected Patients

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ABSTRACT

Background: Qualitative urinary organic acid profile analysis with gas chromatography/mass spectrometry is used in the diagnosing and monitoring of organic acid disorders. The aim of this study is to verify organic acid testing using diagnostic sensitivity and specificity in routine clinical laboratory. **Methods:** Cutoff limits of urinary organic acids were determined as the 95th percentile of healthy volunteers' results. Data of patients submitted to our laboratory were evaluated. Out of 900 clinically suspected patients 25 were diagnosed with 9 different organic acid disorders. **Results:** Results of diagnostic compounds of patients with organic acid disorders were higher than cutoff limits. This reflects sensitivity. Median values of organic acids of the patient group with no specific organic acidemia were all below the cutoff limit which reflects the specificity of the test. **Conclusion:** We suggest that routine laboratories should determine their own cutoff limits and evaluate the overall effectiveness for urinary organic acid profile analysis.

KEYWORDS: Organic acid; method verification; GC-MS

INTRODUCTION

Inborn errors of metabolism (IEMs) are a group of disorders that cause mild to severe irreversible mental and/or physical disability, coma and even death. The range of these disorders is quite wide, covering amino acidopathies, urea cycle disorders, organic acidemias and fatty acid oxidation defects [1,2]. Early detection and treatment of affected newborns can significantly reduce the mortality rate and prevent neurological and physical complications. Amino acid and acylcarnitine profile analysis in dried blood samples by Tandem MS and quantitative amino acid analysis are precious laboratory tests used in diagnosis and monitoring of IEMs.

Urinary qualitative organic acid profile analysis by gas chromatography/mass spectrometry (GC-MS) is a newer technique used for diagnosis and monitoring for organic acidemias. Recently, GC-MS techniques are more widely used in clinical laboratories, however standardizations for method validation and verification are lacking. There are limited number of studies on quantitation and validation of the analysis for a few specific organic acids using GC-MS in literature [3,4]. However, there is no verification study on the qualitative profile analysis conducted using single internal standard likewise in the routine labs.

We aimed to validate the urinary organic acid profile analysis using diagnostic sensitivity and specificity and for this purpose used the results of healthy volunteers and patients diagnosed with organic acidemias in our laboratory.

MATERIALS AND METHODS

Urine samples were collected into urine culture bottles. Samples were stored at -20^{o}C until processing. Creatinine concentrations were determined by Jaffe's method. Organic acids were quantitated using GC-MS with slight modifications as described earlier [2]. 4-phenyl butyric acid was used as the internal standard. Organic acids were extracted with ethyl acetate. Derivatization of organic acids was accomplished by BSTFA and trimethylsilyl chloride.

One microliter of the derivatized organic acids was injected into the GC-MS. Helium was used as the carrier gas. The analysis was performed on a gas chromatograph coupled to a mass spectrometer (GC-MSQP2010SE, Shimadzu, Kyoto, Japan) equipped with an automatic injection system. The data was processed using GC-MS solution software and GC/MS metabolite mass spectral database. The results were calculated from the peak area ratios of the target compounds to the internal standard. Concentrations were normalized to the creatinine concentration of the urine sample and expressed as mmol/mol creatinine.

A series of n-alkane standards were run under the standard method conditions and retention indices were calculated. Tuning and blank analysis with BSTFA and trimethylsilyl chloride were performed prior to the analysis. Concentrations higher than 10mmol/L were considered for reporting. A normal urine sample was analyzed five times and the precision was between 7-22% for diagnostic compounds. Proficiency testing scheme for qualitative urinary organic acid analysis was obtained from ERNDIM and the performance was very good.

Cutoff values of organic acids were set above the 95th percentile of normal subjects, using healthy volunteers' results (n:10) [1,5]. Retrospective data from laboratory information system were examined. Out of 900 clinically suspected patients admitted to Metabolic Diseases Laboratory of Dokuz Eylul University Hospital in 2013, 25 patients were diagnosed with 9 different organic acid disorders. The diagnosis of the disorders were confirmed with clinical evidence, routine laboratory tests, amino acid analysis and tandem MS analysis in addition to organic acid analysis according to standard protocols and/or professional guidelines in all cases. For a few cases DNA analysis were performed.

RESULTS

Methylmalonic acidemia (MMA) was the most common disorder (n=9) followed by glutaric acidemia (GA, n=5). Of the other patients 3 were diagnosed as Alkaptonuria, 2 as 3-methylglutaconic aciduria (MGA), 2 as propionic acidemia (PA), and 1 case each of isovaleric acidemia (IVA), tyrosinemia type 1 and 2 (TYR1 and TYR2) and medium chain fatty acid dehydrogenase deficiency (MCAD).

For these 9 diseases the mean, range and cutoff values of diagnostic compounds calculated using healthy volunteers' results and listed in Table 1. Of these compounds, 8 organic acids could not be detected in healthy volunteers' urine.

Table 1. Cutoff values of organic acids determined by healthy volunteers' urine

Disease	Diagnostic compound	n=10 (mmol/mol creatinine)		
		Mean	Range	Cutoff
MMA	Methylmalonic acid	1.4	0.5-3.8	3.9
	3 hydroxypropionic acid	0.39	0.19-0.79	0.79
	Methylcitric acid	-	-	-
	Propionylglycine	-	-	-
GA	Glutaric acid	0.27	0.06-1.25	1.3
	Glutaconic acid	-	-	-
Alkaptonuria	Homogentisic acid	-	-	-
MGA	3 methylglutaconic acid	3.6	0.32-5.5	12
	3 methylglutaric acid	0.66	0.12-2.5	2.5
IVA	Isovalerylglycine	-	-	-
PA	Propionylglycine	-	-	-
	3 hydroxypropionic acid	0.39	0.19-0.79	0.79
	Metilcitric acid	-	-	-
TYR1	Succinylacetone	-	-	-
TYR2	N acetyltyrosine	0.04	0-0.41	0.41
MCAD	Hexanoylglycine	0.16	0-0.77	0.77

*Indicates not detected, GA, glutaric academia; IVA, isovaleric academia; MCAD, medium chain fatty acid dehydrogenase deficiency; MGA, 3-methylglutaconic aciduria; MMA, methylmalonic acidemia (MMA); PA, propionic academia; TYR1, tyrosinemia type 1; TYR2, tyrosinemia type 2

In the diagnostic approach of suspected cases, most of the patients were presented with hallmarks of acute metabolic decompensation. Lactic acidosis and hyperammonemia were detected in all patients with MMA and PA. Metabolic acidosis with ketosis was mostly common in other patients except MCAD and alkaptonuria. Results of diagnostic compounds of patients with organic acid disorders are shown in Table 2.

Among patients diagnosed with organic acidemias, all diagnostic compounds exceeded the cutoff values, except one. In a patient diagnosed with PA, propionylglycine was below the cutoff value. However, 3-hydroxypropionic acid and metilcitric acid results were above the cutoff values. Therefore, the patient was diagnosed as PA. Methylcitric acid and propionylglycine could not be quantified in six patients having MMA, yet the overall profile of other diagnostic compounds pointed to diagnosis in these patients.

Afterwards, we examined the rest of the organic acid results of our laboratory. Median values of 875 patients not having organic academia diagnosis were lower than the cutoff values. All eight organic acids not quantified in healthy volunteers' urine, were also negative in this group. In addition, N-acetyltyrosine and hexanoylglycine could not be quantified.

Table 2: Urinary analysis of organic acids in the study population

Disease	Diagnostic compound	Patients with organic acidemia		All patients median (n=875) (mmol/mol creatinine)
		n	Mean (Range) (mmol/mol creatinine)	
MMA	Methylmalonic acid 3 hydroxypropionic acid Methylcitric acid Propionylglycine	9	1998 (82-8982) 407 (1.2-188) 97 (0-824) 2.2 (0-14.7)	3.7 0.42 0 0
GA	Glutaric acid Glutaconic acid	5	798 (48-1542) 5.1 (1.2-17)	1.2 0
Alkaptonuria	Homogentisic acid	3	7807 (924-20025)	0
MGA	3 methylglutaconic acid 3 methylglutaric acid	2	985 (69-1901) 126 (6.2-246)	5.2 0.7
IVA	Isovalerylglycine	1	716	0
PA	Propionylglycine 3 hydroxypropionic acid Metilcitric acid	2	13.7 (0-27) 56 (4.5-107) 446 (64-827)	0 0.42 0
TYR1	Succinylacetone	1	13.5	0
TYR2	N acetyltyrosine	1	12	0
MCAD	Hexanoylglycine	1	94	0

GA, glutaric academia; IVA, isovaleric academia; MCAD, medium chain fatty acid dehydrogenase deficiency; MGA, 3-methylglutaconic aciduria; MMA, methylmalonic acidemia (MMA); PA, propionic academia; TYR1, tyrosinemia type 1; TYR2, tyrosinemia type 2

DISCUSSION

Inherited metabolic diseases are caused by genetic defects in metabolic pathways leading to the accumulation or deficiency of related metabolites and damage to organs [6]. Many IEMs have no specific clinical features and the diagnosis is difficult with conventional laboratory tests [7]. Acylcarnitine analysis with Tandem MS and quantitative amino acid analysis are basic metabolic tests used in diagnosis for IEMs. Organic acid analysis is the key diagnostic tool especially for alkaptonuria. In addition, differential diagnosis of MMA from PA is made by comparing the urinary organic acid profiles with GC-MS [8]. The purpose of this study was to gain practical experience in using the new technology and to evaluate the overall effectiveness of the test.

Method validation parameters are clearly specified for GC-MS [4,9]. American College of Medical Genetics (ACMG) revised Standards and Guidelines for Clinical Genetics Laboratories in 2008. Method validation parameters such as tuning the mass spectrometer, linear range, precision, recovery, instrument calibration and quantitation are identified for organic acid analysis. On the other hand, ACMG reported that, clinically meaningful interpretation of organic acid results should be based on the overall pattern of metabolites present in abnormal quantities, rather than on individual abnormal values.

It should be held in mind that simultaneous analysis of urinary organic acids provides a wide range of metabolite profiling, and thus is very important for the diagnosis of organic acidemias [5]. In an organic acid analysis more than 80 organic acids are determined and there is no chance to quantify all of them using internal standards. For this reason, quantitation with at least one internal standard is preferred in routine laboratory practice.

National Association of Testing Authorities (NATA) in Australia published guidelines for validation and verification of quantitative and qualitative test methods. NATA proposes that, bias and precision, sensitivity and specificity should also be considered as performance characteristics for method verification of qualitative tests in addition to matrix effects.

In recent years there are an increasing number of publications about urinary organic acids with increasing clinical use of GC-MS [6,10,11]. Given the inherited nature prevalence of various IEMs are different in populations of diverse ethnical backgrounds [10]. In our study population MMA and GA were the most common disorders similarly reported from other countries [7,12]. MCAD, which is the most common disorder in western countries, was rare in our study population compatible with China, Lebanon and Egypt [6,7,10]. Related to the consanguinity and high birth rate, the frequency of IEMs is high in our country.

In the literature, disease cutoff ranges for amino acid and acylcarnitine analysis with Tandem MS are described in various populations and different values were obtained [1,10,11]. The cutoff limit is described as a clinically defined value between the normal population and a cumulative disease range. This is the first study from the metabolic laboratories in Turkey evaluating the cut off values of organic acids based on their patient populations.

According to our results, concentrations of diagnostic compounds in 25 patients for nine disorders were higher than our cutoff limits. This indicates the sensitivity of the test. The median values of organic acid results of the patients who were not diagnosed with a specific organic acidemia were all below the cutoff limit for each diagnostic compound. This represents the specificity. Sensitivity and specificity reflect the overall effectiveness of qualitative organic acid profile analysis. Organic acid disorders are rare, thus it will be useful to assess laboratory data for organic acid cutoff limits at established intervals in routine laboratories.

CONCLUSION

The evaluation of the accumulating data showed that urinary organic acid profile analysis has satisfactory diagnostic effectiveness for organic acid disorders in our patient population.

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